Intervals from norgestomet withdrawal and injection of equine chorionic gonadotropin or P.G. 600 to estrus and ovulation in ewes

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Intervals from norgestomet withdrawal and injection of equine chorionic gonadotropin or P.G. 600 to estrus and ovulation in ewes[1]

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ABSTRACT: Synchronization of estrus and ovulation is essential for AI of ewes during a predetermined time frame, and progestogen-eCG treatments are typically used to prepare the ewes. However, eCG is not readily available in the United States, but P.G. 600 (400 IU of eCG and 200 IU of hCG) is available. Thus, we conducted a study to determine the effects of eCG and P.G. 600 on the timing of estrus and ovulation after progestogen withdrawal. Ewes were assigned to two replicates of an experiment with the following treatments: 1) 3-mg norgestomet implant (i.e., one-half of a Syncro-Mate-B [SMB] implant) for 10 d, plus 2 mL of saline i.m. at SMB removal (n = 11); 2) 3-mg SMB implant for 10 d, plus 400 IU of eCG i.m. at SMB removal (n = 13); and 3) 3-mg SMB implant for 10 d, plus P.G. 600 i.m. at implant removal (n = 9). On d 6 after SMB insertion, $PGF_{2\alpha}$ was used to induce luteolysis. Beginning 12 h after implant removal, vasectomized rams were used at 12-h intervals to check for estrus. When a ewe was detected in estrus, each ovary was evaluated ultrasonically. Ovaries were evaluated again 16 h later and then at 8-h intervals until ovulation. Treatment altered the interval from implant removal to estrus (less [$P < 0.05$] in SMB + eCG than in the other two groups) and to ovulation (greatest [$P < 0.05$] in SMB). However, the treatment × replicate interaction was significant for the intervals from implant removal to estrus ($P < 0.03$) and from implant removal to ovulation ($P < 0.05$). An inconsistent response in the SMB-treated ewes seemed to be primarily responsible for the interaction. The intervals to estrus and to ovulation for the SMB-treated ewes were shorter ($P < 0.05$) in Replicate 1 than in Replicate 2. Also, both intervals seemed to be less consistent between replicates for the SMB + P.G. 600- than for the SMB + eCG-treated ewes; that is, eCG seemed to increase the predictability of the intervals to estrus and to ovulation. Neither the main effects of treatment and replicate nor their interaction were significant for the interval from estrus to ovulation (38.4 ± 3.3 h), size of the ovulatory follicle (7.7 ± 0.8 mm), or ovulation rate (1.6 ± 0.2). We concluded from this experiment that eCG is a better choice than P.G. 600 as the gonadotropin to use at the time of progestogen withdrawal to prepare ewes for AI during a predetermined interval.

Key Words: Estrus, Gonadotropins, Ovulation, Progestogens, Sheep, Synchronization

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Introduction

Synchronizing estrus and, thus, ovulation creates an opportunity to AI groups of ewes with frozen-thawed semen from superior rams and to increase the overall efficiency of genetic improvement programs. Because $PGF_{2\alpha}$ is only effective in ewes with active corpora lutea, progestogen treatment for 10 to 14 d and an injection of gonadotropin, usually eCG, at progestogen withdrawal has become the most widely used and versatile method for synchronizing estrus in sheep. With this synchronization method, lambing rates after timed laparoscopic AI may average 70% (Rodriguez et al., 1993).

Despite the efficacy and safety of the progestogen-eCG method, the U.S. Food and Drug Administration has not approved the procedure for sheep, and this has severely limited the use of this technology in the United States. Norgestomet implants are approved for synchronizing estrus in cattle, they have been readily available, and they are effective in sheep (Spitzer and Carpenter, 1981; Woody et al., 1983). However, reliable and affordable eCG has not been readily available

[1]The use of trade, firm, or corporation names in this publication is for the information and convenience of the reader. Such use does not constitute an official endorsement or approval by the United States Department of Agriculture or the Agricultural Research Service of any product or service to the exclusion of others that may be suitable. Part of the information in the article was reported in J. Anim. Sci. 76(Suppl. 1):25 (Abstr.).

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in the United States, although a product (P.G. 600; Intervet, Millsboro, DE) containing 400 IU of eCG and 200 IU of hCG is approved for inducing estrus in gilts.

P.G. 600 can be used instead of eCG to prepare ewes for natural breeding (lambing rates of 36 to 59%; Safranski et al., 1992; Jabbar et al., 1994), but, in our experience (unpublished data), P.G. 600 was not suitable for timed laparoscopic AI. Pregnancy rate was very poor in P.G. 600-treated ewes, but fertilization rate was approximately 75% (Maxwell et al., 1996) in ewes superovulated with eCG and FSH that were inseminated at the same time. Based on our data, oocyte quality per se would not seem to have been responsible for the poor pregnancy rate, although AI at an inappropriate time relative to ovulation might have been. Thus, we conducted a study to determine the effects of eCG and P.G. 600 on the timing of estrus and ovulation after progestogen withdrawal.

Materials and Methods

Suffolk × (½ Dorset × ¼ Rambouillet × ¼ Finnsheep) or Dorset × (½ Dorset × ¼ Rambouillet × ¼ Finnsheep) ewes of various ages and in good body condition were used for an experiment that was replicated in the autumn and winter of the same breeding season. Ewes received the following treatments: 1) a 3-mg norgestomet implant (i.e., one-half of a 6-mg Syncro-Mate-B [**SMB**] implant; Rhone Merieux, Athens, GA) inserted s.c. on the posterior aspect of an ear and removed 10 d later, plus a 2-mL i.m. injection of physiological saline given at implant removal (n = 11); 2) 3-mg norgestomet implant for 10 d, plus an i.m. injection of 400 IU of eCG (PMSG; Sioux Biochemical, Sioux City, IA) at implant removal (n = 13); and 3) 3-mg norgestomet implant for 10 d, plus an i.m. injection of P.G. 600 (400 IU of eCG and 200 IU of hCG) at implant removal (n = 9). Six days after implant insertion, $PGF_{2\alpha}$ was injected i.m. (5 mg + 5 mg 4 h later; Lutalyse, Pharmacia & Upjohn, Kalamazoo, MI) into all ewes to induce luteolysis and to prevent existing corpora lutea from affecting the interval from SMB removal to ovulation. Treatments were randomized in blocks, and, as they became available, ewes were assigned to the randomized treatments. For each replicate, seven ewes were allocated for each treatment group. Different ewes were used for each replicate. Each treatment was supposed to be represented in each block. However, even though we adhered to this plan for assigning ewes to groups, some of the ewes that were assigned to groups were not detected in estrus, and only the ewes detected in estrus were evaluated further. Thus, the numbers of ewes evaluated per group were not equal.

Beginning 12 h after implant removal, vasectomized rams were used at 12-h intervals to check for estrus. When a ewe was detected in estrus, she was positioned in dorsal recumbency in a Poldenvale Commodore cradle (Premier Sheep Supplies, Washington, IA), and each ovary was evaluated using a transrectal ultrasonographic procedure (Schrick et al., 1993). An Aloka 500V instrument with a 5.0-MHz transducer was used (Corometrics Medical Systems, Wallingford, CT). Polyvinyl chloride tubing (1.4 cm i.d., 2 cm o.d., and 30 cm long) was used to sheathe the cable, to hold the transducer in a fixed position, and to provide a means for manipulating the transducer in the rectum. Ovaries were evaluated again 16 h after the initial ultrasonographic evaluation and then at 8-h intervals until there was evidence of ovulation. That is, the appearance of the largest follicle(s) changed from a uniform dark gray, which is associated with a fluid-filled structure, to mottled shades of gray with a faint outline of the original structure. The caliper function in the instrument was used to measure the sizes of the largest follicles (i.e., greatest distance across a follicle) at each evaluation, and ovulation rate was determined.

Intervals from implant removal to estrus and to ovulation were estimated. Because estrus detection was at 12-h intervals, the onset of estrus was assumed to have occurred midway between two checks for estrus. Thus, the interval from implant removal to detection of estrus minus 6 h was the value that was estimated for a ewe. Because ultrasound evaluations around the time of ovulation were at 8-h intervals, the interval from implant removal to detection of ovulation minus 4 h was the estimated value. The interval from estrus to ovulation was calculated from these two estimates. The significance of treatment, replicate, and the treatment × replicate interaction for the intervals from 1) implant removal to estrus, 2) implant removal to ovulation, and 3) estrus to ovulation, 4) the size of the ovulatory follicle(s), and 5) ovulation rate was determined with the GLM procedures of SAS (SAS Inst. Inc., Cary, NC). When *F*-values were significant, Tukey's Studentized Range procedure was used to compare the means. The MANOVA/PRINTE options in SAS were used to derive the residual correlation between the interval from implant removal to estrus and implant removal to ovulation and the residual correlation between the interval from estrus to ovulation and the interval from implant removal to ovulation.

Results

The main effect of treatment was significant for the intervals from implant removal to estrus ($P < 0.01$) and from implant removal to ovulation ($P < 0.07$). The interval from implant removal to estrus was less ($P < 0.05$) in the SMB + eCG- than in the SMB- or SMB + P.G. 600-treated ewes, which had similar intervals (Table 1; Figure 1). The interval from implant removal to ovulation was less ($P < 0.05$) for SMB + eCG- than for SMB-treated ewes, and this interval for SMB + P.G.600-treated ewes was intermediate and not different from the intervals for the other two groups (Table 1). In addition, the durations of the ranges for the

Table 1. Interval from norgestomet implant removal to estrus and from estrus to ovulation[a]

Variable	Overall values[b] SMB	SMB + eCG	SMB + P.G. 600	Pooled SE
Implant removal to estrus, h	49.8[c] 36–72	36.1[d] 24–48	47.2[c] 24–60	3.2
Implant removal to ovulation, h	89.4[c] 64–116	75.6[d] 60–96	83.2[cd] 60–112	4.4

[a]Ewes received the following treatments: 1) a 3-mg norgestomet implant (i.e., one-half of a 6-mg Syncro-Mate-B [SMB] implant) for 10 d, plus a 2-mL i.m. injection of physiological saline given at implant removal (n = 11); 2) 3-mg norgestomet implant for 10 d, plus an i.m. injection of 400 IU of eCG at implant removal (n = 13); and 3) 3-mg norgestomet implant for 10 d, plus an i.m. injection of P.G. 600 (400 IU of eCG and 200 IU of hCG) at implant removal (n = 9). Six days after implant insertion, $PGF_{2\alpha}$ was injected i.m. into all ewes to induce luteolysis.

[b]Values are least squares means (above) and ranges (below).

[c,d]Within a row, means without a common superscript differ ($P < 0.05$).

intervals from implant removal to estrus and from implant removal to ovulation seemed to be less for the eCG-treated ewes (24 and 36 h, respectively; i.e., 48 – 24 h and 96 – 60 h, respectively) than for the ewes in the other two groups (36 and 52 h, respectively, for both groups; Table 1).

However, the treatment × replicate interaction was significant for the intervals from implant removal to estrus ($P < 0.03$) and from implant removal to ovulation ($P < 0.05$). An inconsistent response in the SMB-treated ewes seemed to be primarily responsible for the interaction (Table 2). Both intervals for the SMB-treated ewes were considerably shorter ($P < 0.05$) in Replicate 1 than in Replicate 2. Moreover, both intervals seemed to be less consistent between replicates for the SMB + P.G. 600- than for the SMB + eCG-treated ewes (Table 2). The differences between replicates in the average intervals from implant removal to estrus and from implant removal to ovulation were 10.4 and 10.4 h, respectively (i.e., 52.4 – 42.0 h and 88.4 – 78.0 h, respectively), for the SMB + P.G. 600-treated ewes and only 0.2 and 3.8 h, respectively, for the SMB + eCG-treated ewes (Table 2). For the SMB-treated ewes, the differences between replicates for the two intervals were 15.7 and 21.3 h, respectively. Thus, even though the treatment × replicate interaction was significant, the main effect of treatment seems to better illustrate the responses one might expect to the treatments.

Neither the main effects of treatment and replicate nor their interaction were significant for the interval from estrus to ovulation (overall 38.4 ± 3.3 h), size of the ovulatory follicle (overall 7.7 ± 0.8 mm), and ovulation rate (overall 1.6 ± 0.2).

Figure 1 shows the intervals from implant removal to estrus and from implant removal to ovulation for each ewe within each treatment group and the average intervals for each group, relative to the typical time for laparoscopic AI. The residual correlation between the interval from implant removal to estrus and implant removal to ovulation was 0.67 ($P < 0.001$), and the residual correlation between the interval from estrus to ovulation and the interval from implant removal to ovulation was 0.66 ($P < 0.001$). Because of the lack of independence of the variables measured (e.g., the interval from implant removal to estrus is contained within the interval from implant removal to ovulation), the biological relevance of these correlations is difficult to determine. However, they seem to indicate that the time of estrus can be used to reasonably predict the time of ovulation, and, as already described, this interval was consistent among treatments.

Discussion

Treatment with P.G. 600 (400 IU of eCG and 200 IU of hCG) at the time of SMB implant removal had little effect, compared with SMB alone, on the average interval from implant removal to estrus and the average interval from implant removal to ovulation in ewes. By contrast, 400 IU of eCG at the time of SMB removal reduced the intervals to estrus (compared with SMB or SMB + P.G. 600) and to ovulation (compared with SMB). In addition, and perhaps more importantly, eCG seemed to increase the predictability (i.e., reduce the variation in response) of the intervals from implant removal to estrus and from implant removal to ovulation.

The intervals from SMB removal to estrus and to ovulation in our study are similar to those reported previously after either SMB or other progestogen treatments (Spitzer and Carpenter, 1981; Cardwell et al., 1998; Husein et al., 1998). Also, the intervals from SMB removal to estrus and to ovulation in the ewes treated with either eCG or P.G. 600 at the time of progestogen withdrawal are similar to those in previous reports (Quirke et al., 1979 [laparoscopy of groups

of ewes at defined times to determine time of ovulation]; Cardwell et al., 1998 [serial ultrasonography used to determine time of ovulation]). Even though these intervals seem similar among reports, the specific intervals vary considerably among studies. For example, the intervals from progestogen withdrawal and eCG or P.G. 600 treatment to ovulation in the present study were approximately 17 h greater than those reported in Quirke et al. (1979) and 17 h greater than in a study we conducted with a group of ewes with a different genetic background (unpublished data). However, they are within 10 h of the intervals reported in Cardwell et al. (1998). Differences in specific intervals may reflect differences among studies in the breeds or types of sheep used and the experimental protocols and environments. Differences also indicate that one should be cautious about extrapolating across controlled conditions and from controlled to production environments, such as AI in commercial flocks of sheep. Despite the specific differences among studies, eCG at the time of progestogen withdrawal generally increases the predictability of the intervals to estrus and to ovulation (Quirke et al., 1979; Cardwell et al., 1998; present study).

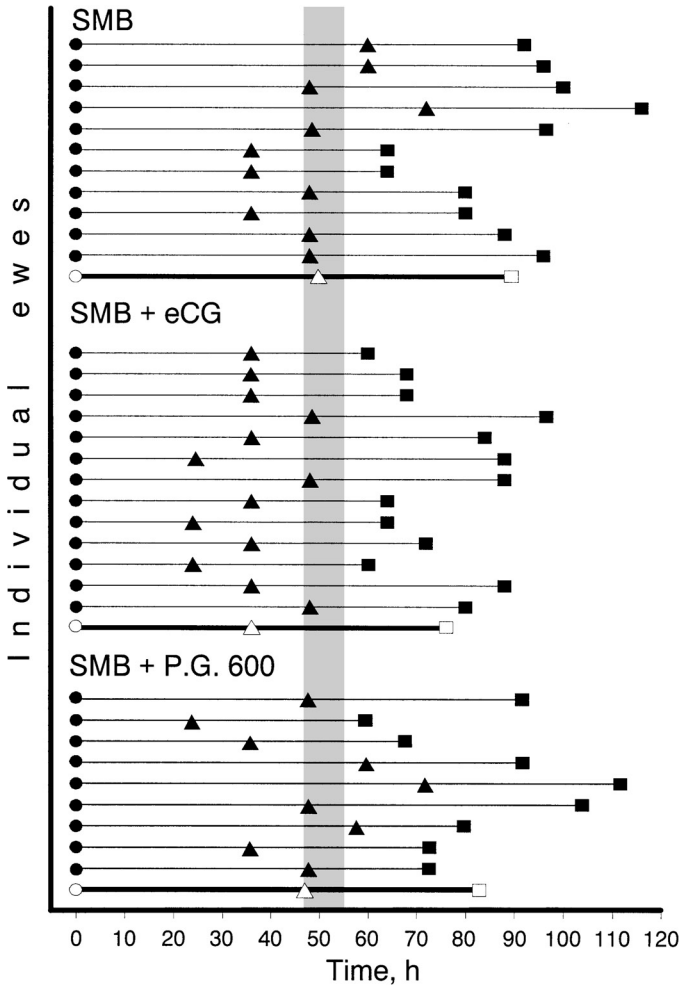

Figure 1. Intervals from implant removal to estrus and from implant removal to ovulation for each ewe (black symbols) within each treatment group and average intervals (open symbols) for each group. The circles indicate when a 3-mg norgestomet implant (i.e., one-half of a 6-mg Syncro-Mate-B [SMB] implant) was removed and either saline (designated SMB), 400 IU of eCG (SMB + eCG), or P.G. 600 (400 IU of eCG and 200 IU of hCG; SMB + P.G. 600) was injected i.m. The triangles indicate when estrus was first detected, and the squares indicate the time of ovulation, which was estimated from periodic transrectal ultrasound examinations. The vertical bar illustrates the typical time (i.e., 48 to 54 h after progestogen withdrawal) for laparoscopic AI.

Sheep are typically inseminated with frozen-thawed semen during a predetermined interval after progestogen withdrawal and eCG treatment. However, the variation among studies in the intervals after progestogen withdrawal and eCG treatment to estrus and to ovulation has made it difficult to settle on a specific time for AI with frozen-thawed semen. Despite that, acceptable fertilization (up to 95%; Jabbour and Evans, 1991; Maxwell et al., 1993) and pregnancy rates (up to 80%; Fukui et al., 1989; Findlater et al., 1991; Husein et al., 1996) are possible after AI during a rather large time frame of 42 to 60 h after progestogen withdrawal and eCG treatment, and this generally encompasses the interval from approximately 24 h before until approximately 5 h after ovulation, although this relationship also varies a good deal among studies. Even though a "best" time for AI with frozen-thawed semen has not been identified, collectively the studies support the idea that sperm should spend enough time in the female reproductive tract to become capacitated and capable of fertilization, without becoming aged. Therefore, gonadotropin treatments that increase the predictability of the intervals from progestogen withdrawal to estrus and to ovulation should increase the chances of conception after AI during a predetermined interval.

Based on results from our study, P.G. 600 did not seem to improve the predictability of the intervals from progestogen withdrawal to estrus and to ovulation. In fact, P.G. 600 seemed to reduce the predictability somewhat, compared with SMB + eCG. This may help explain why the pregnancy rate was so poor (unpublished data) in a group of ewes that we artificially inseminated with frozen-thawed semen during a predetermined interval after progestogen withdrawal and P.G. 600 treatment. It may also help explain why lambing rates after out-of-season breeding were reasonable (i.e., 36 to 59%; Safranski et al., 1992; Jabbar et al., 1994) in ewes that were mated with rams after progestogen withdrawal and P.G. 600 treatment, although Safranski et al. (1992) did not find P.G. 600 to be of any benefit to progestogen treatment. In Jabbar et al. (1994), rams detected estrus, based on crayon marks, and the rams probably inseminated each ewe more than once with large numbers of spermatozoa. By contrast, a single AI with a comparatively small number

Table 2. Interval from norgestomet implant removal to estrus and from estrus to ovulation[a]

Variable	Replicate 1[b] SMB	Replicate 1[b] SMB + eCG	Replicate 1[b] SMB + P.G. 600	Replicate 2[b] SMB	Replicate 2[b] SMB + eCG	Replicate 2[b] SMB + P.G. 600	Pooled SE
Implant removal to estrus, h	42.0[cd] 36–48	36.0[d] 24–48	52.4[ce] 36–72	57.7[e] 48–72	36.2[d] 24–48	42.0[cd] 24–60	3.2
Implant removal to ovulation, h	78.7[c] 64–96	73.7[c] 60–88	88.4[cd] 73–112	100.0[d] 92–116	77.5[c] 60–96	78.0[c] 60–92	4.4

[a]Ewes received the following treatments: 1) a 3-mg norgestomet implant (i.e., one-half of a 6-mg Syncro-Mate-B [SMB] implant) for 10 d, plus a 2-mL i.m. injection of physiological saline given at implant removal (n = 11); 2) 3-mg norgestomet implant for 10 d, plus an i.m. injection of 400 IU of eCG at implant removal (n = 13); and 3) 3-mg norgestomet implant for 10 d, plus an i.m. injection of P.G. 600 (400 IU of eCG and 200 IU of hCG) at implant removal (n = 9). Six days after implant insertion, $PGF_{2\alpha}$ was injected i.m. into all ewes to induce luteolysis.

[b]Values are least squares means (above) and ranges (below).

[c,d,e]Within a row, means without a common superscript differ ($P < 0.05$).

of frozen-thawed, probably damaged, spermatozoa must be as strategic as possible to be successful. Data from the present study indicate that eCG treatment at the time of progestogen withdrawal is more likely than is P.G. 600 treatment to increase the predictability of the intervals to estrus and to ovulation.

We concluded from this experiment that eCG is a better choice than P.G. 600 as the gonadotropin to use at the time of progestogen withdrawal to prepare ewes for AI during a predetermined time frame. We recognize that this conclusion is based on data from a relatively small number of ewes (i.e., 9 to 13 per group), compared with, for example, the number (i.e., 60 to 66 per group) in Quirke et al. (1979), and we did not conduct a fertility trial. However, our conclusion is consistent with the data in Quirke et al. (1979) and Safranski et al. (1992). Therefore, until considerably more research is conducted to determine whether the packaged dose of P.G. 600 and injecting P.G. 600 at the time of progestogen withdrawal are appropriate, we recommend that P.G. 600 should not be used to prepare ewes for AI during a predetermined time frame. However, P.G. 600 may be useful when ewes are to be mated with rams.

Implications

Synchronizing estrus and, thus, ovulation is a critical component of artificial insemination (AI) programs for sheep. This allows sheep to be inseminated with frozen-thawed semen at predetermined times. The success of timed AI programs partly depends on the predictability of the responses of ewes (i.e., intervals from the end of the synchronization process to estrus and to ovulation) to the gonadotropin that is used in a progestogen-based estrus synchronization program. Based on available data, equine chorionic gonadotropin (eCG) seems to increase the predictability of those intervals, whereas P.G. 600, which contains eCG and human chorionic gonadotropin, does not seem to increase the predictability. Therefore, we recommend using eCG, rather than P.G. 600, at the time of progestogen withdrawal to prepare ewes for AI.

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